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Element concentrations of renal and hepatic cells under potassium depletion

FRANZ BECK, ADOLF DÖRGE, JUNE MASON, ROGER RICK, and KLAUS THURAU

Department of Physiology, University of Munich, Munich, Federal Republic of Germany

Element concentrations of renal and hepatic cells under potassium depletion. The effect of dietary potassium depletion on nuclear and cytoplasmic element concentrations in cortical renal tubular cells and hepatocytes was investigated using electron microprobe analysis. Significant differences in sodium and potassium concentrations between nucleus and cytoplasm were not detected either under control or under potassium-depleted conditions. Potassium depletion for at least 14 days resulted in a decrease in plasma potassium concentration from 4.4 ± 0.1 to 2.0 ± 0.1 mmoles·liter^{-1}. There was a fall in cellular potassium from 151.6 ± 3.5 to 120.2 ± 2.1 in distal tubular cells, from 150.1 ± 2.6 to 117.7 ± 1.2 in proximal tubular cells, and from 140.6 ± 1.3 to 128.0 ± 1.3 mmoles·kg^{-1} of wet wt in hepatocytes. The cellular chlorine concentrations fell from 19.9 ± 0.7 to 15.8 ± 0.3 and from 21.3 ± 0.4 to 17.2 ± 0.4 in proximal tubular and liver cells, respectively, but remained unchanged at 11.4 ± 0.7 and 11.0 ± 0.4 mmoles·kg^{-1} of wet wt in distal tubular cells. The intracellular sodium concentrations rose from 10.4 ± 0.7 to 15.8 ± 0.8, 19.1 ± 0.8 to 24.1 ± 0.7 and 14.1 ± 0.5 to 16.2 ± 0.6 mmoles·kg^{-1} of wet wt in distal tubular, proximal tubular and liver cells, respectively. This rise in cellular sodium was insufficient in any cell type to compensate for the loss of potassium. No significant differences were found in the cellular electrolyte concentrations of the various distal tubular cell types which are thought to be involved in either potassium reabsorption or secretion. The decrease in potassium concentrations in distal tubular cells by about 20% does not seem sufficient to explain the marked fall in urinary potassium excretion.

Concentration en éléments des cellules rénales et hépatiques lors de la déplétion potassique. L'effet de la déplétion potassique alimentaire sur les concentrations en éléments nucléaires et cytoplasmiques des cellules tubulaires rénales corticales et les hépatocytes a été étudié en utilisant au moyen de la microsonde électronique. Aucune différence dans les concentrations de sodium et de potassium entre le noyau et le cytoplasme n'a été détectée aussi bien dans les conditions contrôles qu'après déplétion potassique. Une déplétion potassique d'au moins 14 jours a entraîné une diminution de la concentration plasmatique de potassium de 4,4 ± 0,1 à 2,0 ± 0,1 mmoles·litre^{-1}. Il s'est produit une chute du potassium cellulaire de 151,6 ± 3,5 à 120,2 ± 2,1 dans les cellules tubulaires distales, de 150,1 ± 2,6 à 117,7 ± 1,2 dans les cellules tubulaires proximales et de 140,6 ± 1,3 à 128,0 ± 1,3 mmoles·kg^{-1} de tissu humide dans les hépatocytes. Les concentrations de chlore cellulaire ont diminué de 19,9 ± 0,7 à 15,8 ± 0,3 et de 21,3 ± 0,4 à 17,2 ± 0,4 dans les cellules tubulaires proximales et hépatiques respectivement, mais sont restées inchangées à 11,4 ± 0,7 et 11,0 ± 0,4 mmoles·kg^{-1} de tissu humide dans les cellules tubulaires distales. Les concentrations de sodium intracellulaire se sont élevées de 10,4 ± 0,7 à 15,8 ± 0,8, de 19,1 ± 0,8 à 24,1 ± 0,7, et de 14,1 ± 0,5 à 16,2 ± 0,6 mmoles·kg^{-1} de tissu humide dans les cellules tubulaires distales et proximales, et hépatiques respectivement. Cette élévation du sodium cellulaire n'était suffisante dans aucune des cellules pour compenser la perte en potassium. Aucune différence significative dans les concentrations cellulaires en électrolytes n'a été trouvée entre les divers types de cellules tubulaires distales qu'on pense être impliquées dans la réabsorption ou la sécrétion potassique. La diminution des concentrations en potassium dans les cellules tubulaires distales d'environ 20% ne semble pas suffisante pour expliquer la chute marquée de l'excrétion urinaire de potassium.

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It is well established that the distal tubular segment of the kidney regulates urinary potassium excretion and thereby stabilizes the potassium content of the body. Micropuncture experiments [1, 2] have demonstrated that the function of the distal tubule to regulate potassium excretion is accomplished by the dual process of secretion and reabsorption. As shown by many investigators, an impoverishment of the body potassium is accompanied by a drastic reduction in urinary potassium excretion [1–4]. The reduced potassium excretion during severe potassium depletion results from a decrease in potassium secretion [1, 3] and an increase in potassium reabsorption [2, 4] in distal tubular segments. On the basis of a three-compartment analysis of the potassium secretory process, it was concluded that the intracellular potassium concentration is the major factor in determining the potassium secretory rate in the distal tubule [5]. This view is supported by determinations of the total renal content of potassium [4, 6, 7] and cellular potassium activities in the distal tubule [8], both of which showed a decrease in renal cellular potassium after potassium depletion. However, as the potassium contents obtained by chemical analyses represent only mean values of the whole renal tissue, the changes in the potassium concentration of distal tubular cells following potassium depletion cannot be derived. Because, in addition, owing to technical difficulties, activity measurements in renal tubular cells have given conflicting values [8–10], it seems opportune to reexamine the cellular potassium concentrations in distal tubular cells after potassium depletion.

In this investigation the technique of electron microprobe analysis was used to measure the intracellular electrolyte concentrations following potassium depletion. Compared to the use of ion selective electrodes or chemical analyses, this method offers the advantage that the analysis can be performed in individual, well-defined cells. This feature of the method might be of special importance, because it is assumed that potassium secretion and reabsorption in the distal tubule involve various epithelial cell types [11] that may react differently

to a dietary potassium deficiency [12]. A further advantage of electron microprobe analysis is that many cellular elements can be detected simultaneously, enabling intracellular changes in the potassium concentrations to be correlated to alterations in the other electrolytes of the cell.

The measurements were performed in renal distal and proximal tubular cells and liver cells of the rat. The analyses in proximal tubular cells and liver cells were performed to clarify whether or not these cell types exhibit a similar alteration in their electrolyte concentrations after potassium depletion as those cells which are involved in the regulation of potassium excretion, namely the distal tubular cells.

Methods

Dietary pretreatment. The experiments were performed on 34 male Sprague-Dawley rats weighing between 180 and 270 g and given free access to food and water. The animals were maintained on either a control diet or a low potassium diet and demineralized water for 14 to 22 days. The control diet (Altromin C 1000) and the low potassium diet (Altromin C 1037) were casein diets of similar composition, both containing 237 mmoles·kg^{-1} sodium and 136 chloride, but differing mainly in potassium composition, which was 116 mmoles·kg^{-1} in the control diet and less than 3 mmoles·kg^{-1} in the low potassium diet.

Preparation of the animals. Kidney experiments. The rats were placed in metabolic cages the day before the experiments commenced and urine samples were collected under oil for 24 hr to determine urinary volume, sodium, potassium and chloride concentrations and osmolality. Anesthesia was achieved with 100 to 120 mg·100 g body wt^{-1} inactin i.p. (Byk-Gulden, Konstanz, West Germany). The animals were placed on a heated operation table which allowed body temperature to be maintained between 37 and 38° C. The trachea was cannulated. Both the right jugular vein and the right femoral artery were catheterized with polyethylene tubing for the infusion of saline and to enable blood pressure to be monitored and blood samples to be obtained. The left kidney was exposed through a flank incision, freed from adherent fat and connective tissue, and placed into a plastic cup (Lucite™). The ureter was cannulated close to the renal pelvis to collect urine samples. Mineral oil, heated to 38° C, was dropped on the exposed kidney surface throughout the experiments. For the determination of glomerular filtration rate, 3g·100 ml^{-1} polyfructosan (Inutest, Laevosan) dissolved in isotonic saline was given via the jugular catheter as 0.2 to 0.3 ml·100g body wt^{-1} initially, followed by 0.3 to 0.5 ml·hr^{-1}·100 g body wt^{-1} constant infusion. Urine samples were collected for 30-min intervals for the determination of filtration rate, sodium, potassium, and chloride concentrations; blood samples were taken at the midpoint of the collection periods. At the end of the clearance period the kidney surface was carefully blotted free of paraffin oil and covered with a thin layer of albumin Ringer's solution. The whole kidney then was removed rapidly from the animal, shock-frozen in propane cooled to −180° C, and stored subsequently in liquid nitrogen.

Liver experiments. The animals were anesthetized with ether, and the liver was exposed through a midline incision. A small part of the liver lying on the surface was covered with a thin layer of albumin Ringer's solution. This portion was then removed rapidly, shock-frozen in liquid propane at −180° C, and stored in liquid nitrogen. A blood sample was taken from the abdominal aorta into a heparinized syringe subsequently for analysis of the sodium, potassium, and chloride concentrations and the osmolality.

Electron microprobe analysis of tissue samples. While immersed in liquid nitrogen the frozen tissue was fractured into small pieces with a precooled scalpel blade. Small pieces from the surface of the tissue, containing an adherent albumin standard, were sandwiched between two pliable indium foils and then firmly mounted into a clamp-type holder. Approximately 1-μm thick sections were cut in a cryomicrotome (Reichert Om U 2) at −80° C and freeze-dried at −80° C and 10^{-6} Torr. Electron microprobe analysis was performed in a scanning electron microscope (Cambridge S4) using an energy dispersive x-ray detector system (LINK) at an acceleration voltage of 17 kV and a probe current of 0.5 nA. Areas of about 1 μ^2 were analyzed for 100 sec in the energy range between 0.6 and 4.0 keV. Quantification of element concentrations and dry weight was achieved by comparing the element characteristic radiations and the white radiation with those of the internal standard. The standard albumin Ringer's solution, which contained 20 g bovine albumin·100 g^{-1} and 140, 110, and 4.5 mmoles·kg^{-1} of wet wt sodium, chloride, and potassium, respectively, was adapted to the plasma potassium concentration in the low-potassium animals by reducing the potassium concentration to 2 mmoles·kg^{-1} of wet wt. Details of the preparation of freeze-dried cryosections for microanalysis and the quantification procedure have been described [13–15]. The cellular measurements were confined mostly to the nuclei of the tissue, but in some cases additional measurements were also made in the cytoplasm. Care was taken in the renal tubular cells, characterized by large basolateral infoldings and brush borders, to analyze only those cytoplasmic areas known to contain nearly no extracellular space [16].

Chemical analysis of fluid samples. Sodium and potassium concentrations in urine, plasma, and standard solutions were determined by flame photometry (IL), chloride concentrations by chloridometry (Eppendorf). Osmolality was measured from the depression of freezing-point (Vogel, Giefsen/Lahn, West Germany) or vapor-pressure (Wescor Inc., Logan, Utah). The polyfructosan concentration in urine and plasma was determined photometrically using the anthrone method [17]. The dry weight of the standard solutions was determined by drying to constant weight at 100° C.

Statistical evaluation of the data. The intracellular element concentrations, expressed in mmoles·kg^{-1} wet wt, were pooled for each element and experimental group. The data are presented as the mean ± SEM, with the number of observations in parentheses. Statistical evaluation of the results was performed with the Student's t test for related or unrelated samples, as appropriate. Differences were considered to be statistically significant, if the two-tailed probability was 0.05 or less.

Results

Plasma electrolytes and renal function. The values obtained for the plasma and renal status of the animals after 14 to 22 days on a control or potassium-deplete diet are summarized in Table 1. Examination of the blood plasma showed a significant fall in the potassium concentration during potassium depletion,

Table 1. Plasma composition, urinary excretion, and renal function under control conditions and following potassium depletion in rats

	Control	*N*	K-depleted	*N*
P_K, *mmoles·liter*$^{-1}$				
P_{Osm}, *mOsm·kg*$^{-1}$	294 ± 3	*12*	293 ± 2	*22*
P_K, *mmoles·liter*$^{-1}$	4.4 ± 0.1		2.0 ± 0.1[a]	
P_{Na}, *mmoles·liter*$^{-1}$	141.8 ± 1.1		139.0 ± 0.9	
P_{Cl}, *mmoles·liter*$^{-1}$	97.6 ± 0.6		92.6 ± 1.2[a]	
Urinary excretion				
V, *ml·24 hr*$^{-1}$	11.2 ± 1.2	*5*	44.3 ± 4.5[a]	*10*
U_{Osm}, *mOsm·kg*$^{-1}$	1593 ± 224		496 ± 49[a]	
U_KV, *mmoles·24 hr*$^{-1}$	1.3 ± 0.1		0.08 ± 0.02[a]	
$U_{Na}V$, *mmoles·24 hr*$^{-1}$	3.14 ± 0.3		2.35 ± 0.25	
$U_{Cl}V$, *mmoles·24 hr*$^{-1}$	2.04 ± 0.19		1.77 ± 0.16	
Renal function				
V, *μl·min*$^{-1}$*·100 g BW*$^{-1}$	0.6 ± 0.1	*5*	2.2 ± 0.6[a]	*10*
U/P_{Inulin}	722 ± 83		206 ± 40[a]	
GFR, *ml·min*$^{-1}$*·100 g BW*$^{-1}$	0.34 ± 0.03		0.33 ± 0.04	
U_K, *mmoles·liter*$^{-1}$	214.8 ± 51.4		2.3 ± 0.5[a]	

N represents the number of animals.
[a] This value is statistically different from the corresponding control value.

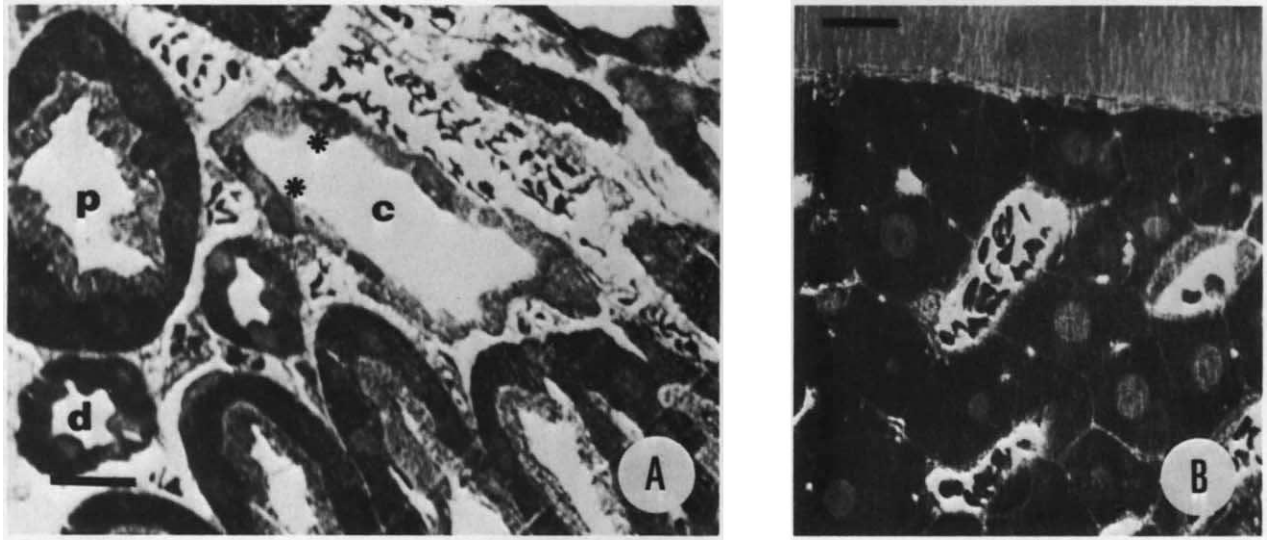

Fig. 1. *Scanning transmission electron micrographs of freeze-dried cryosections of rat renal cortex* **(A)** *and rat liver* **(B)**. Abbreviations are: p, proximal convoluted tubule; d, distal convoluted tubule; c, cortical collecting tubule. Two dark appearing cells in the cortical collecting tubule are marked with *asterisks*. The homogeneous layer at the surface of the liver **(B)** represents the albumin standard. Bar equals 10 μ.

whereas the osmolality, sodium concentration, and chloride concentration were almost unchanged. For the animals involved in the renal studies, the production of urine in the 24 hr preceding anesthesia was found to be enhanced by potassium depletion and the urinary osmolality to be depressed. The excretion of potassium was lowered to 6% of that observed in control rats, whereas the excretion of sodium and chloride was almost unaffected. Compared to control conditions, the inulin clearance measured during anesthesia was unaffected by dietary potassium depletion. However, the casein diet fed to both control and potassium-depleted rats produced a fall in GFR of about 20% compared to that found in previous studies [16, 18], in which the rats were fed a standard diet (Altromin C 1320). As in the 24-hr collection period the urinary volume was increased and the potassium concentration decreased. This increase in the urinary volume was accompanied by a reduced U/P inulin ratio.

Tissue morphology. Figure 1 shows scanning transmission electron micrographs of freeze-dried cryosections of renal cortex and liver, both from control animals. In the kidney section (Fig. 1**A**) the prominent brush border characterizes the proximal tubule (p), and its absence allows the distal tubules to be discerned. Among the distal tubules, the distal convolution (d) and collecting tubule (c) can be differentiated. The cells of the distal convolution are of one type, whereas those of the collecting tubule are mixed, comprising a lighter and a darker cell type. In the liver section (Fig 1**B**), the erythrocyte-filled sinusoids are immediately apparent. Two types of cells can be distinguished, the hepatocytes and the cells lining the sinusoids.

Table 2. Nuclear element concentrations and dry weight in distal and proximal tubular cells and in hepatocytes under control conditions and following potassium depletion[a]

	N	K	Na	Mg	Cl	P	Ca	Dry weight
		mmoles·kg^{-1} of wet wt						*g·100 g^{-1}*
Distal tubule								
Control	31 (5)	151.6 ± 3.5	10.4 ± 0.7	14.5 ± 0.8	11.4 ± 0.7	193.0 ± 5.0	0.2 ± 0.1[c]	19.1 ± 0.5
K-depleted	93 (10)	120.2 ± 2.1[b]	15.8 ± 0.8[b]	12.9 ± 0.7	11.0 ± 0.4	185.5 ± 2.8	0.2 ± 0.1[c]	19.7 ± 0.4
Proximal tubule								
Control	73 (5)	150.1 ± 2.6	19.1 ± 0.8	14.3 ± 0.4	19.9 ± 0.7	152.8 ± 3.1	0.2 ± 0.1[c]	21.3 ± 0.4
K-depleted	224 (10)	117.7 ± 1.2[b]	24.1 ± 0.7[b]	13.4 ± 0.6	15.8 ± 0.3[b]	144.8 ± 1.6[b]	0.2 ± 0.1[c]	21.7 ± 0.3
Hepatocytes								
Control	126 (7)	140.6 ± 1.3	14.1 ± 0.5	11.8 ± 0.6	21.3 ± 0.4	132.0 ± 1.8	0.3 ± 0.1	24.0 ± 0.3
K-depleted	148 (12)	128.0 ± 1.3[b]	16.2 ± 0.6[b]	10.8 ± 0.4	17.2 ± 0.4[b]	133.1 ± 1.9	0.2 ± 0.1[c]	23.8 ± 0.3

N equals total number of measurements; number of animals is given in parenthesis.
[a] Data are presented as mean ± SEM.
[b] Value is statistically different from corresponding control value.
[c] Value is statistically not different from zero.

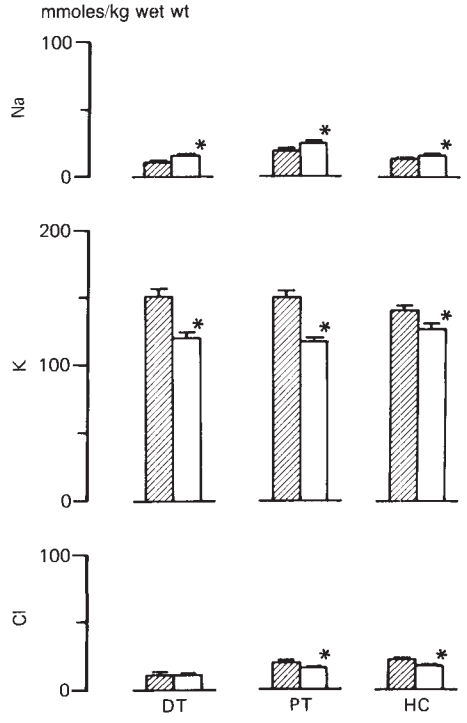

Fig. 2. *Nuclear sodium, potassium, and chlorine concentrations under control conditions* (hatched columns) *and following potassium depletion* (open columns) *in distal tubules* (DT), *proximal tubules* (PT), *and hepatocytes* (HC). Data are given as mean ± 2 SEM. *Asterisk* represents a value statistically different from the corresponding control value.

Intracellular element concentrations following potassium depletion. Table 2 lists the nuclear element concentrations of proximal and distal cortical kidney tubules and hepatocytes in control and following potassium depletion. In addition, for all three cell types the changes in the sodium, potassium, and chlorine concentrations are illustrated in Figure 2. The data for the distal tubule comprise measurements in cells of distal convolutions and in light and dark cells of collecting tubules, as no difference in the cellular electrolyte composition could be discerned between these cell types, either in control or potassium-depleted rats.

In distal tubular cells the potassium concentration fell from 152 to 120 mmoles·kg^{-1} of wet wt following potassium depletion, while the sodium concentration increased from 10 to 16 mmoles·kg^{-1} of wet wt, and the chlorine concentration remained constant at 11 mmoles·kg^{-1} of wet wt. The phosphorus, magnesium, and calcium concentrations and the dry weight showed only very small, mostly statistically insignificant variations. Similar results were observed in proximal tubular cells and in hepatocytes. In the proximal tubule the potassium concentration dropped from 150 to 118 mmoles·kg^{-1} of wet wt, while the sodium concentration increased from 19 to 24 mmoles·kg^{-1} of wet wt. In addition, a small drop in the chlorine concentration from 20 to 16 mmoles·kg^{-1} of wet wt was observed. In hepatocytes, the fall in the potassium concentration following potassium depletion was somewhat less pronounced, from 141 to 128 mmoles·kg^{-1} of wet wt. Sodium rose from 14 to 16 and chlorine decreased from 21 to 17 mmoles·kg^{-1} of wet wt. Again, in both cell types no significant changes were found in the phosphorus, magnesium, and calcium concentrations and the dry weight after potassium depletion.

Comparison between nuclear and cytoplasmic element concentrations. Table 3 shows the results of a smaller number of paired analyses in the nucleus and cytoplasm of distal and proximal tubular cells and of hepatocytes. In control as well as after potassium depletion, no significant differences between nuclear and cytoplasmic sodium and potassium concentrations were observed. In contrast, systematic differences were detected in the concentration of chlorine, phosphorus, and dry weight which in all three cell types were slightly higher in the cytoplasm than in the nucleus.

Discussion

Maintaining rats for 14 to 22 days on a low potassium diet resulted in a drastic reduction of the plasma potassium concentration of 2.5 mmoles·liter^{-1} and a moderate but significant decrease in plasma chloride concentration of about 5 mmoles·liter^{-1}, whereas plasma sodium concentration and os-

Table 3. Nuclear and cytoplasmic element concentrations and dry weight in distal and proximal tubular cells and in hepatocytes under control conditions and following potassium depletion[a]

	N	K	Na	Cl	P	Dry weight
		mmoles·kg^{-1} of wet wt				*g·100 g^{-1}*
Distal tubule						
Control						
Nucleus	11 (5)	148.4 ± 4.9	9.8 ± 0.9	10.1 ± 0.4	188.2 ± 6.5	19.0 ± 0.5
Cytoplasm		143.3 ± 5.0	9.7 ± 0.8	17.2 ± 0.9[b]	238.0 ± 10.0[b]	26.9 ± 0.9[b]
K-depleted						
Nucleus	34 (10)	119.1 ± 3.0	15.6 ± 1.0	10.5 ± 0.6	182.1 ± 4.4	19.1 ± 0.5
Cytoplasm		119.5 ± 3.1	15.7 ± 0.9	16.5 ± 1.1[b]	217.9 ± 4.8[b]	25.3 ± 0.8[b]
Proximal tubule						
Control						
Nucleus	24 (5)	149.0 ± 4.3	19.2 ± 1.3	19.2 ± 0.7	151.4 ± 5.2	21.1 ± 0.5
Cytoplasm		142.3 ± 4.7	18.0 ± 1.2	33.7 ± 1.7[b]	193.0 ± 6.7[b]	30.4 ± 0.9[b]
K-depleted						
Nucleus	51 (10)	116.7 ± 2.5	24.7 ± 1.8	14.8 ± 0.5	145.7 ± 3.7	21.7 ± 0.5
Cytoplasm		111.4 ± 3.1	23.2 ± 1.7	27.7 ± 1.1[b]	185.5 ± 4.1[b]	29.9 ± 0.7[b]
Hepatocytes						
Control						
Nucleus	46 (7)	140.2 ± 1.9	13.9 ± 0.7	22.1 ± 0.5	132.3 ± 2.4	24.3 ± 0.4
Cytoplasm		136.4 ± 2.0	12.4 ± 1.1	25.2 ± 0.7[b]	173.1 ± 5.4[b]	33.2 ± 0.7[b]
K-depleted						
Nucleus	66 (12)	126.4 ± 1.9	16.0 ± 0.5	16.9 ± 0.6	132.8 ± 2.4	23.5 ± 0.4
Cytoplasm		122.9 ± 2.0	14.5 ± 0.9	21.9 ± 0.7[b]	160.6 ± 5.2[b]	32.6 ± 0.5[b]

N equals total number of paired measurements; number of animals is given in parenthesis.
[a] Data are presented as mean ± SEM.
[b] Value is statistically different from corresponding nuclear value.

molality remained unchanged. Similar changes in plasma potassium concentration have been observed after a comparable dietary treatment [1, 2, 5, 7]. The small decrease in plasma chloride concentration might indicate a hypochloremic metabolic alkalosis, often observed after prolonged potassium depletion [19–22]. Further characteristic features of potassium deprivation are a drastic reduction in renal potassium excretion [1–4] and an impaired concentrating ability of the kidney [23]. In this study the potassium excretion decreased from about 9 to less than 1% of the filtered potassium load. The inability of the kidney to concentrate the urine is indicated by a fourfold increase in the urinary volume and an almost equivalent decrease in urinary osmolality.

The cellular element concentrations obtained in this study under control conditions in the kidney are very similar to those reported in previous studies [16, 18]. This is valid for both the absolute values observed in the proximal and distal tubular cells and the differences between these tubular segments. That microprobe analysis reveals smaller sodium and higher potassium concentrations in renal tubular cells than most of the chemical analyses might be due to an underestimation of the extracellular compartment by the latter method. This point has been discussed extensively in previous reports [16, 18, 24]. Electron microprobe measurements of intracellular element concentrations in hepatocytes, however, are in reasonable agreement with chemical analyses [25] and with measurements of potassium and chloride activities [26]. The absence of a concentration difference between nucleus and cytoplasm for sodium and potassium in both kidney cells and hepatocytes under control and potassium-depleted conditions is in good agreement with activity measurements [27] and electron microprobe analyses [24, 28] of other epithelial cells. However, in contrast to the present results, large concentration differences for these ions were reported between nucleus and cytoplasm in fractionated liver cells using chemical analysis [29].

It is generally accepted that regulation of renal potassium excretion is accomplished mainly by distal tubular segments. According to the current concept of distal tubular potassium transport [30], potassium secretion involves active uptake of potassium in exchange for sodium across the peritubular cell membrane and passive efflux across the luminal membrane, while potassium reabsorption involves active uptake across the luminal membrane and passive efflux across the peritubular membrane. The luminal membrane is depolarized markedly and has a high permeability for potassium.

The reduction in renal potassium excretion induced by potassium depletion can be explained by a decrease in passive potassium efflux and/or stimulation of active uptake across the luminal cell membrane. It is conceivable that a decreased potassium concentration in distal tubular cells might be responsible for the fall in the passive potassium efflux across the luminal membrane, as postulated by de Mello-Aires, Giebisch, and Malnic [5]. Using radiochemical analysis these authors found a decreased cellular potassium transport pool following potassium depletion but virtually no alteration of the rate constants for the potassium fluxes across the luminal cell membrane. Such a mechanism could contribute substantially to the decrease in urinary potassium excretion following potassi-

um deprivation. However, the present data do not support this concept since the 20% fall in the cellular potassium concentration observed is not nearly adequate to explain the 15-fold decrease in urinary potassium excretion. Furthermore, if the concentration gradients for potassium across the luminal cell membrane are considered, the chemical driving force for potassium efflux might even be enhanced because the potassium concentration of the tubular fluid is reduced markedly under potassium depletion [1–3, 8]. This interpretation, however, presumes that almost no potassium is in a bound form within the renal tubular cells. A final decision regarding this point as yet is not possible because firstly, the potassium concentrations measured in this study differ markedly from the potassium activities reported for rat renal tubular cells and secondly, intracellular activity coefficients for potassium are not known. The cellular potassium concentration, as calculated from the present data on the basis of cellular water, is about 190 mmoles·liter^{-1}, whereas potassium activities of about 40 [8, 10] and more recently of 82 mmoles·liter^{-1} [9] have been reported. Whether this means that substantial amounts of the intracellular potassium are bound in the renal epithelium or that the intracellular potassium activity coefficient is much smaller than in extracellular fluids and other cell types, such as frog skin [31] and hepatocytes [26], remains to be clarified. In the frog skin, the difference between the potassium activity as determined by ion selective electrodes [31] and the potassium concentration measured by electron microprobe analysis [24] can be explained fully by an activity coefficient typical for extracellular fluids [31], suggesting there is very little potassium binding in these cells. This then could raise the question of whether the potassium activities determined by ion selective electrodes in rat renal cells is falsely low or the potassium concentrations as measured by electron microprobe analysis in these cells are falsely high. An overestimation of the intracellular potassium concentration by electron microprobe analysis seems very unlikely since all possible artifacts occurring during the preparation and analysis of the tissue should lead to a redistribution of the intra- and extracellular electrolytes [32] and hence to a decrease in intracellular potassium concentration.

Two further factors which can influence the passive potassium efflux across the luminal membrane are either changes in membrane potential or potassium permeability. However, information regarding these parameters in normal and depleted rats is scarce and controversial. There is agreement that following potassium depletion the transepithelial potential difference in the distal tubule is lowered, but it remains unclear whether this is due to hyperpolarization of the luminal membrane [8], which would inhibit potassium efflux, or depolarization of the basolateral membrane [33]. To date, no direct measurements of the potassium permeability of the luminal membrane have been undertaken during potassium depletion. Alternatively, active reabsorption of potassium may be instrumental in the regulation of renal potassium excretion. A recent microelectrode study performed on the Amphiuma kidney [34] has provided some evidence that a reduced active luminal potassium uptake in the distal tubule is in part responsible for the increased urinary potassium excretion during potassium adaptation.

Based upon morphological alterations in the cell types of the late distal tubule during changes in the potassium intake [35–40], it has been proposed that the principal cells are involved in potassium secretion, while the intercalated cells are engaged in potassium reabsorption [12]. The present experiments, which revealed no variations in cellular electrolyte composition in the distal tubular cells as might be expected for cells with differing transport properties, supply no additional information with which to evaluate this hypothesis.

The changes in the electrolyte composition in proximal tubular cells and liver cells after potassium depletion were found to be very similar to those in the distal tubular cells, suggesting that the fall in the intracellular potassium concentration in the distal tubule merely reflects the same potassium loss observed in other tissues. Qualitatively, the same changes have been reported already for skeletal muscle [4, 6, 7, 19].

The fact that the loss of cellular potassium during potassium depletion is not accompanied by an equivalent gain in cellular sodium, a phenomenon known as "cation deficit," has been the subject of many investigations [21]. The ratio sodium gain/potassium loss which has been found to be 0.6 to 0.7 in muscle cells [7, 19, 21, 41] is much smaller in this study and amounts to 0.15 for hepatocytes and 0.2 for kidney cells. This cationic deficit following potassium depletion might be balanced by an increase in cellular calcium and magnesium [41], an increase in basic amino acids [42, 43] and/or a fall in cellular pH [19, 22, 44, 45]. However, in the present experiments a compensation of the cationic deficit by an increase in intracellular calcium and magnesium can be excluded, as the concentration of magnesium is virtually unchanged during potassium depletion. In addition the nuclear calcium concentration under both experimental conditions amounts to less than 0.5 mmoles·kg^{-1} of wet wt.

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Reprint requests to Dr. F. Beck, Physiologisches Institut, Pettenkoferstrasse 12, D-8000 München 2, Federal Republic of Germany

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